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(57) Abstract

System, method and device for promoting growth of tissue regenerate into a wound area in an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction. An encasement structure (5) encases the wound area to inhibit ingress of granulation tissue to the wound area and mechanical guide means (11) for the outgrowing tissue regenerate are disposed in the encased wound area so as to extend in the predetermined direction. In one aspect a fibrin network formation inhibiting agent is concomitantly administered to the wound surface of the encased wound area. In another aspect the mechanical guide means takes the form of a gel structure provided with one or more guide channels for the outgrowing tissue regenerate which extend in the predetermined direction.

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PROMOTION OF REGENERATION OF ORGANIZED TISSUES

The present invention relates to the promotion of regeneration of organised tissue in a wound area in an organised tissue structure of a living human or animal body, such as for example nerves (spinal and cranial), tendons, ligaments, skeletal muscle, bone, joint capsules, cartilage and aponeuroses.

It is to be noted that the term "regeneration" and derivatives thereof mentioned herein are not to be taken as necessarily meaning the repair of a wound area in an organised tissue structure by the formation of replacement organised tissue in the wound area which is identical to the original organised tissue but simply repair of the wound area by the formation of replacement organised tissue in the wound area *per se*.

The repair of wound areas in organised tissue structures after traumas resulting from surgical procedures or injuries such as transection, crushing or otherwise is hampered due to incomplete restoration of both structure and function. In the case of nerve repair and regeneration several implant structures have been previously proposed to address this problem.

For example, the use of guide filaments for the repair of severed nerves has hitherto been suggested (Alexander et al: Proc. Soc. Exp. Biol. Med. 68: 380-383, (1948); Stopford: Lancet, 10 1296-1297, (1920)). This typically consists of threading a suture across the gap between the proximal and distal ends of the severed nerve. It has also been made known to coat the guide filaments with materials such as laminin, collagen and fibronectin. The use of guide filaments, however, has met with limited success.

The use of an open-ended tube for bridging the proximal and distal ends of a severed nerve to promote the regeneration of nerve tissue across the space between the proximal and distal ends of the nerve has also been documented (Glück: Arch. Klin. Chir. 25: 606-616, (1880); Forssman: Ziegler's Beiträge zur Pathol. Anatomie 27: 407 (1900)). The use of such

a nerve guide tube results in an increase in the number and/or size of regenerating axons and a decrease in the time required for the regenerating nerve tissue to bridge the damaged region. The provision of a collagen gel matrix in the lumen of a nerve guide tube has been shown to further improve the regeneration rate of the nerve tissue in the gap between the proximal and distal ends of the severed nerve (T. Satou et al: Acta pathologica Japonica 36, 199-208, 1986 and Acta pathologica Japonica 38(12), 1489-1502, 1988).

It has further been proposed in Canadian patent No. 1328710 (Aebischer et al) to form a nerve guide tube with a non-porous outer surface and a porous inner surface so that a nerve growth-inducing active factor can be incorporated therein for slow release thereof into the wound area. Similarly, International patent application publication No. WO92/13579 (Fidia S.P.A.) discloses a biodegradable and bioabsorbable guide tube for the repair and regeneration of nerve tissue in which a growth factor is supported in the boundary wall of the lumen to enhance nerve regeneration, growth and repair.

The incorporation of mechanical guide structures in the lumen of a nerve guide tube has also been suggested. For instance, International patent application publication No. WO88/06871 (Astra Meditec) makes known providing a plurality of axially extending guide channels in the lumen of an open-ended tube for entubation of the distal and proximal ends of a severed nerve. The guide channels are defined between and/or through fibres which extend axially in the tube lumen. A nerve guide tube having a plurality of isolated lumens formed by laser drilling a plurality of bores through a cylinder body is also envisioned.

Detracting factors in the use of entubation for the repair of damaged nerves, though, such as extensive inflammation and/or compression of the nerve, have led some experts in the field to conclude that the only damaged nerve regions successfully bridged by entubation techniques are those which could be more appropriately closed (Sunderland: Peripheral Nerve Injuries and Their Repair, p. 605 (1978) and Nerve Injuries and Their Repair, Churchill Livingstone, p. 431 ff (1991)).

The use of guide tubes has also been suggested for the regeneration of other body structures. For instance, International patent application publication No. WO88/06872 (Astra Meditec) makes known an implant structure for promoting the regeneration of tendons, ligaments and cruciate ligaments comprising an open-ended tube into the lumen of which the free end of a torn tendon, ligament or cruciate ligament is inserted and through which a plurality of tissue guiding channels extend axially. The guide channels in the tube lumen are defined by the spacing between filaments or members which extend axially through the lumen.

None of the hitherto proposed structures, though, take the network of fibrin and cells including platelets (hereinafter the "fibrin network") inevitably formed at the surface of an injured tissue structure into account.

The Applicant, on the other hand, has appreciated the significance that the fibrin network plays in the repair and regeneration process of organised tissues. Outgrowing cells such as regenerating tissue structures demand a physical support for their adhesion and migration. In the case of regenerating tissue in a traumatised area this mechanical supporting structure is provided or determined, in the formative growth stages at any event, by the fibrin network established in the traumatised area as the backbone of a clot. The effect of this is that the structure of the fibrin network exerts crucial influence on the direction of the cells invading the injured area both with regard to the invading granulation tissue cells and the specific cells characterising the healing structure, that is to say, the fibrin network constitutes a template for the direction and distribution of cells characterising the healing structure in a traumatised area.

For instance, in the case of an injured nerve the track for the regenerating axons and the supporting Schwann cells across a gap or a crushed area of a nerve is largely related to the distribution and organisation of the complex fibrin network. Tendons, ligaments, aponeuroses, skeletal muscle, cartilage, bone and other organised tissue structures all show

a similar dependence on the pattern created by the fibrin network in the clot filling substance after injury.

With this in mind, the fibrin network formed in a traumatised area of an organised tissue structure has a highly complex, irregular 3-D structure of branched fibrin threads or filaments. Accordingly, in the case of nerve tissue repair the outgrowing Schwann cells and axons advance along the fibrin filaments and branch when the fibrin threads branch or cross each other. The same pattern is true for the connective tissue cells accompanying the nerve regenerate, that is to say, their course largely follows the pattern of the fibrin network filling the gap between the severed nerve ends or the crushed nerve area. This dependence is equally evident in severed structures as after crush injuries or surgical procedures.

The result of this dependence in the case of nerve tissue regeneration is that the impressive high regeneration capacity is severely compromised because the vast majority of outgrowing new axons take a highly aberrant course, branch extensively and fail to take their proper path resulting in the outgrowing new axons being unable to reach their presumed targets and re-establish functional connections. The consequence of the highly branched, misaligned and randomly directed axons in the seemingly healed nerve being unable to reattain innervation of the presumed targets is that neuromas are formed. The outgrowth is thus terminated prematurely with a fraction of the nerve cells eventually becoming lost.

The presence, distribution and organisation of the fibrin network is thus is a key factor in determining the subsequent organisation of the tissue regenerate formed during the repair process and thus the ability of the injured structure to function properly again.

The present invention therefore proposes to enhance wound healing in an organised tissue structure in a living human or animal body by providing means for controlling the direction of tissue regenerate growth.

According to a first aspect of the invention there is provided a system for promoting growth of tissue regenerate into a wound area in an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction comprising an encasement structure adapted in use to be implanted in the living human or animal body to encase the wound area, mechanical guide means for the tissue regenerate adapted in use to be disposed in the encased wound area so as to extend in the predetermined direction, and a fibrin network formation inhibiting agent administrable to the wound surface of the encased wound area. Typically, the fibrin network formation inhibiting agent will be administered to the encased wound area systemically or locally.

It is to be understood that the term "inhibiting" in the feature "fibrin network formation inhibiting agent" covers both the case of inhibition of the formation of a fibrin network in the wound area and also the degradation of a pre-existing fibrin network in the wound area.

According to a second aspect of the invention there is provided a method for promoting growth of tissue regenerate in a wound area of an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction comprising the steps of encasing the wound area with an encasement structure, providing mechanical guide means for the tissue regenerate in the encased wound area such that the mechanical guide means extends in the predetermined direction, and administering a fibrin network formation inhibiting agent to the encased wound area.

In an embodiment of the invention the fibrin network formation inhibiting agent comprises a thrombin inhibitor. The thrombin inhibitor may be a low molecular weight peptide-based thrombin inhibitor. The term "low molecular weight peptide-based thrombin inhibitor" will be well understood by those skilled in the art to include thrombin inhibitors with one to four peptide linkages, and/or with a molecular weight below 1000, and includes those described in the review paper by Claesson in Blood Coagul. Fibrin. (1994) 5, 411 as well as those disclosed in US patent No. 4346078, International patent application publication Nos. WO93/11152, WO95/23609, WO95/35309, WO96/25426, WO94/29336, WO93/18060

and WO95/01168 and European patent application publication Nos. 648780, 468231, 559046, 641779, 185390, 526877, 542525, 195212, 362002, 364344, 530167, 293881, 686642, 669317 and 601459.

Preferred low molecular weight peptide-based thrombin inhibitors include those collectively known as "gatrans", examples being melagatran (HOOC-CH_2-(R)Cgl-Aze-Pab-H: see International patent application publication No. WO94/29336 and the list of abbreviations therein) and inogatran (HOOC-CH_2-(R)Cha-Pic-Nag-H: see International patent application publication No. WO93/11152 and the list of abbreviations therein) .

The thrombin inhibitor may also be a bisulphated polysaccharide or oligosaccharide such as a chondroitin sulphate, a dermatan sulphate, a dextron sulphate, a keratan sulphate, a heparan sulphate or heparin. Alternatively, the thrombin inhibitor may be a hirudin, a biosynthetic analogue of hirudin, a fragment of hirudin such as a fragment consisting of at least the last 8 C-terminal amino acids of the known sequence in hirudin or the protein NAPc2.

In another embodiment of the invention the fibrin network formation inhibiting agent comprises a fibrinolytic agent. The fibrinolytic agent may be a plasminogen activator (tPA), for example a recombinant human plasminogen activator (hrtPA) such as Actilyse®, streptokinase or urokinase

In further embodiments of the invention the fibrin network formation inhibiting agent comprises a Factor X inhibitor, a trypsin inhibitor or a protease inhibitor, that is to say, other compounds which affect the activity of the thrombinogen-thrombin system which instigates the fibrin network formation.

In an embodiment of the invention the fibrin network formation inhibiting agent is immobilised to the inner surface of the encasement structure which in use faces the wound area.

In an embodiment of the invention the fibrin network formation inhibiting agent is in solution and a pump is provided to administer the fibrin network formation inhibiting agent to the encased wound area. The pump may be an osmotic minipump which may further be adapted to be implanted subcutaneously in the living human or animal body.

In an embodiment of the invention the fibrin network formation inhibiting agent is incorporated in a matrix material for disposal or delivery to the encased wound area. As an example, the matrix material may be formed of a material comprising a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, an agar gel, a hydrogel such as methylcellulose gel, Matrigel®, Biomatrix I®, water, saline, phosphate buffered saline, a lipid or a protein such as collagen.

According to a third aspect of the invention there is provided an implantable device for promoting growth of tissue regenerate into a wound area in an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction comprising an outer encasement structure which when the device is implanted in the living human or animal body encases the wound area, and an inner gel structure provided with one or more guide channels for the tissue regenerate which when the device is implanted is disposed in the encased wound area such that the guide channels extend in the predetermined direction. The implantable device may be used in conjunction with a fibrin network formation inhibiting agent in accordance with the invention although this is not strictly necessary.

In an embodiment of the invention the encasement structure is a patch for a crush wound area or the like of the organised tissue structure.

In an alternative embodiment according to the first and second aspects of the invention the encasement structure is a tube having an open end adapted to receive the wound surface and the mechanical guide means is adapted in use to extend in the predetermined direction in the lumen of the tube. If the fibrin network formation inhibiting agent is being

administered locally into the lumen of the tube by infusion from a pump then a uniform distribution of the agent may be promoted by using a tube comprising an outer continuous tube element for inhibiting ingress of granulation tissue to the wound area through which a tube connected to the pump passes and an inner tube element formed of a plurality of longitudinally spaced apart tube sections for axially distributing the agent discharged from the pump into the lumen.

In an alternative embodiment according to the third aspect of the invention the encasement structure is a tube having an open end adapted to receive the wound surface with the or each guide channel extending in the predetermined direction in the lumen of the tube.

In an embodiment of the invention according to the first and second aspects the wound surface of the wound area is a first wound surface, the open end of the tube is a first open end, the tube has a second open end adapted to receive a second wound surface of the wound area and the mechanical guide means is adapted in use to extend in the lumen of the tube between the first and second open ends in the predetermined direction.

In an embodiment of the invention according to the third aspect the wound surface of the wound area is a first wound surface, the open end of the tube is a first open end, the tube has a second open end adapted to receive a second wound surface of the wound area and the or each guide channel extends in the lumen of the tube between the first and second open ends in the predetermined direction.

In these cases the invention can be for promoting growth of tissue regenerate across a gap between the severed or transected free ends of an organised tissue structure such as a nerve, tendon, skeletal muscle or ligament, the open ends of the tube each being adapted to receive one of the severed or transected free ends.

The encasement structure is preferably of a biocompatible material and may be biodegradable or non-biodegradable. Biodegradable is preferred however.

The encasement structure may be constructed from a material comprising a polysaccharide, for example a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

The encasement structure may also be constructed of a material comprising collagen or other protein complexes.

Alternatively, the encasement structure may be constructed from a material comprising a polymer or copolymer, for example polylactic acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, polyglucuronic acid, or poly-N-acetylglucosamine or copolymers thereof such as a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid.

Where the encasement structure is constructed from a non-biodegradable material possible materials are silicone and ethylene-vinyl acetate.

In an embodiment of the invention according to the first and second aspects the mechanical guide means is supported or presented by the inner surface of the encasement structure which in use faces the wound area. In this instance the mechanical guide means and the encasement structure could be integrally formed as an implantable body.

In an embodiment of the invention according to the first and second aspects the mechanical guide means takes the form of guide channels in the encased wound area.

One way to achieve this would be to have an encasement structure which when implanted is a tube-like structure having a transverse spiral cross-section formed for example by rolling up a planar membrane. The guide channels are then defined by the longitudinally extending spaces presented by the spiral cross-section.

Another way is to have mechanical guide means which take the form of a gel structure which is provided with one or more guide channels therethrough, the gel structure adapted

in use to be disposed in the encased wound area such that the guide channels extend in the predetermined direction.

Where the invention is for promoting the growth of nerve tissue regenerate the or each guide channel has a cross-sectional dimension in the range of 50μm-1mm and preferably a cross-sectional dimension in the range of 150-500μm to allow growth of nerve functional units through the channels, that is to say, nerve fascicles. For other organised tissue structures the cross-sectional dimension of the or each guide channel would be chosen to allow growth of the corresponding functional units therethrough.

In an embodiment of the invention the gel structure is formed from agar, a hydrogel such as methylcellulose gel, albumin or other proteins which can be formed into gel, a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, a lipid which can be formed into a gel, Matrigel® or Biomatrix I® .

In another embodiment of the invention according to its first and second aspects the mechanical guide means comprises one or more guide filaments or fibres adapted in use to extend across the encased wound area in the predetermined direction. The mechanical guide means may for example be monofilaments, multifilaments or woven/non-woven fibres.

Preferably, the or each guide filament or fibre is of a biocompatible material which is also a biodegradable material. The or each guide filament or fibre may though be formed from a non-biodegradable material.

In an embodiment of the invention according to the first and second aspects the or each guide filament or fibre is formed from a material comprising a polysaccharide such as a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

In an alternative embodiment of the invention according to the first and second aspects the or each guide filament or fibre is formed from a material comprising a polymer or

copolymer. As examples, the or each guide filament or fibre may be formed from polylactic acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, poly-N-acetylglucosamine or copolymers thereof such as for example a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid.

In another embodiment of the invention according to its first and second aspects the or each guide filament or fibre is formed from collagen or other protein complexes.

In an embodiment of the invention according to the first and second aspects the mechanical guide means comprises one or more suture filaments formed for example from vicryl, catgut, polyamid, chitin or nylon.

Where non-biodegradable guide filaments are to be used silicone is suitable.

In an embodiment of the invention a growth factor or mixture of growth factors may be administered to the encased wound area. The growth factor may for example be immobilised to the inner surface of the encasement structure.

The growth factor may comprise insulin-like growth factors-I, insulin-like growth factors-II, platelet derived growth factors, fibroblast growth factors, transforming growth factors-β, transforming growth factors-α, neurotrophines, ciliary neurotrophic factors, EGF or glial growth factors. The growth factor may also comprises Schwann cells, endothelial cells, fibroblasts, macrophages or inflammatory cells or genetically altered cells which can express a growth factor.

According to a fourth aspect of the invention there is provided use of a system according to the first aspect of the invention for promoting growth of tissue regenerate in a wound area of an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction.

According to a fifth aspect of the invention there is provided use of an implantable device according to the third aspect of the invention for promoting growth of tissue regenerate in a wound area of an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction.

Examples of the organised tissue structures on which the invention can be used for promoting the growth of tissue regenerate in a wound area are nerves, tendons, ligaments, joint capsules, cartilage, bone, aponeuroses and skeletal muscles.

The invention thus provides for inhibiting or controlling the formation of the fibrin network in injured tissue coupled with the provision of mechanical guide means to enable the cells emerging in the wound area from the wound surface to follow the track offered by the mechanical guide means. The advantage of this is that the cells do not show any branching or irregular path as a coherent path for growth is provided. It is therefore possible to achieve regeneration of organised specific cells, such as Schwann cells from peripheral nerves or tenocytes from tendons, to bridge the defect induced by an injury with minimal diversions and irregularities in the organisation of the newly formed tissue.

To illustrate the invention experiments conducted on adult rats under permits O 293/93, O 69/95 and O 70/95 granted by the Animal Experiments Ethical Committee of the University of Gothenburg will now be described with reference to the accompanying Figures of drawings in which:

Fig. 1 is a cross-sectional side view of a guide tube having a lumen into the opposing ends of which the proximal and distal ends of a transected sciatic nerve of an adult rat are sutured and through which a guide filament extends schematically illustrating the fibrin network formed in the lumen between the proximal and distal ends in the absence of administration of a fibrin network formation inhibiting agent.

Fig. 2 is a cross-sectional side view of a guide tube and guide filament assembly corresponding to that of Fig. 1 into the opposing ends of the tube of which the proximal and distal ends of a transected sciatic nerve of an adult rat are sutured schematically illustrating the fibrin network formed with administration of a fibrin network formation inhibiting agent.

Fig. 3 is a cross-sectional side view of a guide tube corresponding to that of Fig. 1 into the lumen of which only the proximal end of a transected sciatic nerve of an adult rat is sutured schematically illustrating the fibrin network formed in the absence of administration of a fibrin network formation inhibiting agent.

Fig. 4 is a cross-sectional side view of a guide tube corresponding to that of Fig. 1 into the lumen of which only the proximal end of a transected sciatic nerve of an adult rat is sutured schematically illustrating the fibrin network formed with administration of a fibrin network formation inhibiting agent.

Fig. 5 is a cross-sectional side view of a guide tube and guide filament assembly corresponding to that of Fig. 1 into the lumen of the tube of which only the proximal end of a transected sciatic nerve of an adult rat is sutured schematically illustrating the fibrin network formed in the absence of administration of a fibrin network formation inhibiting agent.

Fig. 6 is a cross-sectional side view of a guide tube and guide filament assembly corresponding to that of Fig. 1 into the lumen of which only the proximal end of a transected sciatic nerve of an adult rat is sutured schematically illustrating the fibrin network formed with administration of a fibrin network formation inhibiting agent.

Fig. 7 is a cross-sectional side view of a guide tube comprising an outer continuous tube element and an inner tube element formed from longitudinally spaced apart sections having a lumen into one end of which the proximal end of a transected sciatic nerve of an adult rat is sutured and through which a guide filament extends schematically illustrating the fibrin

network formed with local administration of a fibrin network formation inhibiting agent by infusion from an implanted osmotic minipump.

Fig. 8 is a schematic cross-sectional side view of a guide tube having a lumen into which the proximal and distal ends of a transected sciatic nerve of an adult rat are sutured at opposing open ends and which is filled with a gel provided with longitudinally extending guide channels.

Referring to Figs 1 and 2, there is shown a silicone guide tube 5 into the opposite open ends of which the proximal end 1 and the distal end 3 of a transected sciatic nerve of an adult rat have been inserted and fixed in place by sutures 7.

The guide tube 5 prevents or inhibits access of granulation tissue to the wound area and surfaces by isolating the injured nerve structure from the surrounding tissue, usually connective tissue with blood vessels. This makes it easier to control the repair and modelling of the tissue regenerate formed between the severed ends of the injured sciatic nerve . The guide tube 5 further acts as a delivery system for compounds interfering with the formation of a fibrin network in the wound area as will become apparent and may also act as a slow delivery system for growth stimulating agents.

While Figs 1 and 2 show the injured nerve extending only a short distance into the guide tube 5, both the proximal and distal ends 1, 3 may be covered by longer lengths of the guide tube 5. This distance may extend up to the point where division of a nerve fascicle at the distal end prevents further encasement.

A guide filament 11 formed from an ophthalmic suture material extends between the proximal and distal ends 1, 3 of the injured sciatic nerve through the lumen of the guide tube 5. Of course, a plurality of guide filaments can be used instead. When the guide filament 11 is surgically inserted a segment of the filament projects outside the guide tube 5. This segment is then cut away when the surgical repair procedure is completed.

In Fig. 1 the injured sciatic nerve was left to regenerate in the presence of saline introduced into the guide tube 5 at surgery. The result after a few days is a large complex fibrin network 13 of branching filaments formed along the guide filament 11 in the gap between the proximal and distal ends 1, 3 of the sciatic nerve for subsequent regenerating nerve tissue to follow.

As in the Fig. 1 set up, the guide tube 5 in the Fig. 2 set up was also filled with saline at surgery. In addition, however, melagatran was systemically administered to the wound area by subcutaneous infusion with an implanted minipump (not shown). As schematically shown, a narrower fibrin network 14 outlined the central guide filament 11 in the gap between the transected nerve ends. More importantly, the fibrin network 14 exhibited a coherent structure of fibrin platelets and fragments aligned in the direction of the filament 11 for subsequent regenerating nerve tissue to follow.

In Figs 3 and 4 there is shown a silicone guide tube 105 corresponding to the guide tube 5 of Figs 1 and 2. In these set ups, though, only the proximal end 101 of a transected sciatic nerve of an adult rat is held in place in the tube 105 with a suture 107 and no guide filament extends through the lumen. Thus one of the open ends 102 of the tube is left open. As before, saline was introduced into the lumen of the tube 105 at surgery.

In the Fig. 3 set up no further treatment was provided. A tiny clot 115 consisting of fibrin, platelets and other blood cells was formed just covering the proximal end but no fibrin network formed in the rest of the tube. No substantial fibrin network was thus formed to support subsequent nerve tissue regenerate growth.

By contrast, in the Fig. 4 set up melagatran was additionally systemically administered to the wound area by infusion subcutaneously with an implanted minipump. A small clot 116 of fibrin and cells was formed just covering the proximal nerve end. There was no fibrin network in the rest of the tube. No substantial fibrin network was thus formed to support subsequent nerve tissue regenerate growth.

Referring to Figs 5 and 6 there is shown a silicone guide tube 205 corresponding to the guide tube 5 of Figs 1 and 2. As in Figs 3 and 4, the proximal end 201 of a transected sciatic nerve of an adult rat is held in the tube 205 with a suture 207 and the lumen of the tube filled with saline at surgery. In contrast to Figs 3 and 4, though, a guide filament 211 in the form of an ophthalmic suture extends from the proximal end 201 into the lumen.

In the case of Fig. 5 no further treatment was administered and a narrow complex clot 217 consisting of irregularly organised fibrin filaments, platelets and other blood cells was formed along the guiding filament 211 throughout the tube 205 for subsequent regenerating nerve tissue to follow.

In the case of Fig. 6, on the other hand, melagatran was systemically administered to the wound area by infusion subcutaneously with an implanted minipump. A small clot 218 of coherent fibrin platelets, fibrin fragments and cells was formed along and aligned with the guiding filament 211 in the centre of the tube for subsequent regenerating nerve tissue to follow.

In Fig. 7 there is shown an open-ended guide tube 305 comprising an outer continuous tube element 306 and an inner tube element 308 formed from a plurality of longitudinally spaced sections 310. As shown, the proximal end 301 of a transected sciatic nerve of an adult rat is inserted into the tube and maintained in place with a suture 307. A guide filament 311 in the form of an ophthalmic suture extends through the lumen of the tube from the proximal end.

The tube was filled with saline at surgery and then melagatran infused locally to the encased wound area with an implanted osmotic minipump 312. The longitudinally spaced sections 310 assist in distributing the melagatran throughout the gap in the lumen. A narrow coherent clot 319 of coherent fibrin platelets, fibrin fragments and cells was formed along, and aligned with, the length of the guiding filament 311 in the centre of the tube for subsequent regenerating nerve tissue to follow.

The construction of the guide tube shown in Fig. 7 may be varied such that each isolated longitudinal section is provided with one or more guide filaments. The guide tube and filament assembly is thus essentially comprised of smaller individual assemblies of similar construction. This facilitates the separation of regenerating nerve fascicles since the longitudinal sections act as a separate guide for each fascicle

Turning now to Fig. 8 there is shown an open-ended guide tube 405 into the opposing ends of which the proximal and distal ends 401, 403 of a transected or severed sciatic nerve of an adult rat have been inserted and fixed with sutures 407. The lumen of the tube 405 in this case is filled with agar gel 402 through which a plurality of axial guide channels 404 extend. The gel structure 402 is shown spaced from the end surfaces of the transected nerve structure for the sake of clarity although needless to say it is not a strict requirement for the gel structure to abut the nerve end surfaces.

The result of the set up is the formation of a coherent fibrin network in the guide channels having long fibrin filaments or cables axially aligned with the guide channels for subsequent regenerating nerve tissue to follow.

The same systems as described above with reference to the accompanying Figures of drawings, modified with regard to shape and dimensions, have also been tested on tendons, ligaments, abdominal aponeuroses and skeletal muscles of adult rats with corresponding results.

The use of silicone tubes and ophthalmic sutures for the guide filaments was selected in the set ups described hereinabove with reference to the accompanying Figures of drawings only due to the fact that they are suitable for use in experimental animals.

The guide tube may be formed from biocompatible bioresorbable or non-bioresorbable materials which may be permeable or impermeable to materials soluble in aqueous solutions. Use of a bioresorbable or biodegradable material, though, is preferred. Materials of which

the guide tube may be constructed include, but are not limited to, collagen complexes, heparin and heparonoids, chitosan and related polysaccharides, polylactic acid, polyglycolic acid, polyhydroxybutyric acid, permselective polytetraethylene, poly-N-acetylglucosamine, or polymers into which growth factors may be incorporated directly (e.g. ethylene-vinyl acetate).

With regard to the guide thread filaments, these may be formed from biocompatible materials similar or identical to the materials used for the guide tube. Other materials that may be used include presently available suture materials, such as vicryl, catgut, nylon, chitin as well as other materials which can act as a compatible substrate for the formation of a cable of regenerating tissue such as nerve axons.

The invention will now be further illustrated, but in no way limited, by the following examples conducted on adult rats under the ethical permits hereinabove identified and cross-referenced where appropriate with the set ups hereinbove described with reference to the accompanying Figures of drawings.

Example 1

The sciatic nerve of adult rats was exposed unilaterally on the mid thigh and transected. The proximal and distal ends were then inserted into a silicone guide tube of the type shown in Figs 1 to 6 of the accompanying drawings to leave a 10mm gap therebetween filled with phosphate buffered saline (PBS). The silicone tube was sutured to the perineurium of the inserted nerve ends with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were fairly few and showed extensive aberration, often arranged as loops. The Schwann cells were identified in the central parts of the regenerate arranged in a seemingly random pattern. Fibroblasts formed an enclosing perineurium-like

structure. Numerous macrophages were distributed throughout the gap region as were scattered erythrocytes.

Nerve regeneration was thus blocked in absence of a mechanical guiding structure bridging the gap between the nerve ends, such as a fibrin network covered guide filament.

Deficient repair of the nerve was thus achieved

Example 2

The sciatic nerve of adult rats was exposed unilaterally on the mid thigh and transected. The proximal and distal ends were then inserted into a silicone guide tube to leave a 10mm gap therebetween. The silicone tube was sutured to the perineurium of the inserted nerve ends with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

As in the set up hereinabove described with reference to Fig. 1, the gap was filled with PBS and a single central guiding filament (10-0 monofilament nylon) positioned in the lumen of the tube to connect the proximal and distal nerve ends.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons appeared to be slightly more numerous than in Example 1 but still showed extensive aberration, again often arranged as loops. The Schwann cells were identified in the central parts of the regenerate arranged in a seemingly random pattern. Fibroblasts formed an enclosing perineurium-like structure. Numerous macrophages were distributed throughout the gap region as were scattered erythrocytes.

Deficient repair of the nerve was thus achieved due to the nerve tissue regenerate following the path laid by a complex fibrin network formed in the early stages of the repair process.

Example 3

The sciatic nerve of adult rats was exposed unilaterally on the mid thigh and transected. The proximal and distal ends were then inserted 2mm into a silicone guide tube having an inner diameter of 1.5mm to leave a 10mm gap therebetween. The silicone tube was sutured to the perineurium of the inserted nerve ends with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

As in the experiment hereinabove described with reference to Fig. 2, a single central guiding filament (10-0 monofilament nylon) was positioned in the lumen of the tube to connect the proximal and distal nerve ends, the gap was filled with PBS and melagatran infused systemically via the peritoneum with the aid of an osmotic minipump (Alza 2001, volume =220µL; pumping rate = 1 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Melagatran, Astra Hässle AB, Mölndal, Sweden) implanted into the peritoneal cavity for a week and the outlet of the pump positioned free in the peritoneal cavity.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were at least as numerous as in Example 1 and 2, showed rare aberration and were arranged parallel to the central guiding filament, i.e. exhibited a very high degree of coherence. The Schwann cells showed a high degree of coherence being mainly arranged parallel to the central guiding filament and few cells diverged from that direction. There was hardly any random pattern in the organisation of the Schwann cells. Fibroblasts formed an enclosing perineurium-like structure. A striking feature was the absence of macrophages and blood cells outside the regenerate.

Excellent regeneration across a 10 mm gap was thus achieved due to the nerve tissue regenerate following the path laid by a coherent fibrin network formed in the early stages of the repair process.

Example 4

The sciatic nerve of adult rats was exposed unilaterally on the mid thigh and transected. The proximal and distal ends were then inserted 2mm into a silicone guide tube to leave a 10mm gap therebetween. The silicone tube was sutured to the perineurium of the inserted nerve ends with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

As in Example 3, a single central guiding filament (10-0 monofilament nylon) was positioned in the lumen of the tube to connect the proximal and distal nerve ends, the gap was filled with PBS and melagatran administered. In this case, though, a tube from an osmotic minipump (Alza 2002, volume =220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Melagatran, Astra Hässle AB, Mölndal, Sweden) implanted subcutaneously in the back of the animal delivered melagatran solution locally into the gap during at least 8 days in the manner shown in Fig. 7.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were at least as numerous as in Examples 1 to 3, showed rare aberration and were arranged parallel to the central guiding filament, i.e. exhibited a very high degree of coherence. The Schwann cells were mainly arranged parallel to the central guiding filament and few cells diverged from that direction. There was no random pattern in the organisation of the Schwann cells. Fibroblasts formed an enclosing perineurium-like structure. There were no macrophages and blood cells outside the regenerate.

Excellent regeneration of coherent axons across a 10 mm gap was thus again achieved.

Example 5

As in the set up hereinabove described with reference to Fig 3, the sciatic nerve of adult rats was transected and the proximal end inserted into a silicone guide tube filled with PBS.

No regenerate formed from the proximal nerve end after 2 or 4 weeks. Macrophages and blood cells could be recognised close to the proximal nerve end.

Nerve regeneration was thus blocked in the absence of a mechanical guiding structure extending from the proximal nerve end, such as a fibrin network covered guide filament.

Example 6

As in the experiment hereinabove described with reference to Fig 4, the sciatic nerve of adult rats was transected and the proximal end inserted into a silicone guide tube filled with PBS and to which melagatran was infused systemically with the aid of an implanted osmotic minipump.

As in Example 5, though, no regenerate formed from the proximal nerve end after 2 or 4 weeks. Macrophages and blood cells could be recognised close to the proximal nerve end.

Nerve regeneration was thus blocked in the absence of a mechanical guiding structure extending from the proximal nerve end, such as a fibrin network covered guide filament.

Example 7

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. Immediately thereafter the proximal and distal nerve ends were inserted 2 mm into a silicone tube of the type shown in Figs 1 to 6 prefilled with buffered saline. The gap between the nerve ends was 10 mm. The silicone tube was sutured to the perineurium of the inserted

nerve ends with 9-0 "atraumatic" ophthalmic sutures (Ethicon). An osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Melagatran, Astra Hässle AB, Mölndal, Sweden) was implanted subcutaneously in the back of the animal and the outlet of the pump connected via a tube to the mid portion of the silicone tube enclosing the transected nerve for local delivery of melagatran into the gap as shown in Fig. 7.

The nerves and the tube with its gap were examined after 2 and 4 weeks with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised by immunohistochemistry with the aid of antibodies to neurofilaments (N 0142 & N 5389, Sigma) and to the neuroglial protein S-100 (S 2644, Sigma; Z 0311, Dakopatts). There was no regenerate bridging the gap. No axons or Schwann cells traversed the gap. Scattered amorphous protein strands and cells could be recognised in the gap fluid.

Nerve regeneration was thus blocked in the absence of a mechanical guiding structure bridging the gap between the nerve ends, such as a fibrin network covered guide filament.

Example 8

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. Immediately thereafter the proximal and distal nerve ends were inserted 2 mm into a silicone tube of the type shown in Figs 1 to 6 prefilled with buffered saline. However, there was no guiding filament, e.g. a suture thread, in the centre of the silicone tube. The gap between the nerve ends was 10 mm. The silicone tube was sutured to the perineurium of the inserted nerves with 9-0 "atraumatic" ophthalmic sutures (Ethicon). An osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Melagatran, Astra Hässle AB, Mölndal, Sweden) was implanted in the peritoneal cavity and the outlet of the pump left open for systemic delivery of melagatran via the peritoneal cavity and the blood system.

The nerve and the tube with its gap were examined after 2 and 4 weeks with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised by immunohistochemistry with the aid of antibodies to neurofilaments (N 0142 & N 5389, Sigma) and to the neuroglial protein S-100 (S 2644, Sigma; Z 0311, Dakopatts). There was no regenerate bridging the gap. No axons or Schwann cells traversed the gap. Scattered amorphous protein strands and cells could be recognised in the gap fluid.

Nerve regeneration was thus blocked in absence of a mechanical guiding structure bridging the gap between the nerve ends, such as a filament covered with a fibrin network.

Example 9

The sciatic nerve of adult rats was transected and the proximal end inserted 2mm into a silicone guide tube. As in the experiment hereinabove described with reference to Fig. 5, a single central guiding filament (10-0 monofilament nylon) was stitched through the proximal sciatic nerve end to extend into the lumen and the lumen was filled with PBS with the aid of an implanted osmotic minipump. The distal end of the transected sciatic nerve was positioned among the thigh muscles so that it could not interfere with the growth in the silicone tube.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were sticking to the central guiding filament and many appeared to be parallel to it. However, the axons showed extensive aberration and loops were common. The Schwann cells were to a large extent arranged parallel to the central guiding filament but many cells diverged from that direction. Fibroblasts formed an enclosing perineurium-like structure. There were numerous macrophages and blood cells outside the regenerate.

Deficient repair of the nerve was thus achieved due to the nerve tissue regenerate following the path laid by the complex fibrin network formed in the early stages of the repair process.

Example 10

The sciatic nerve of adult rats was transected and the proximal end inserted 2mm into a silicone guide tube. As in the experiment hereinabove described with reference to Fig. 6, a single central guiding filament (10-0 monofilament nylon) was stitched through the proximal sciatic nerve end to extend into the lumen, the lumen was filled with PBS and melagatran then infused systemically with the aid of an implanted osmotic minipump. The distal end of the transected sciatic nerve was positioned among the thigh muscles so that it could not interfere with the growth in the silicone tube.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were arranged parallel to the central guiding filament and very few diverged. There was no axonal aberration and hardly any loops at all. The Schwann cells were to a large extent arranged parallel to the central guiding filament. Fibroblasts formed an enclosing perineurium-like structure. There were rarely any macrophages and blood cells outside the regenerate.

Excellent regeneration of coherent axons was thus achieved due to the nerve tissue regenerate following the path laid by a coherent fibrin network formed in the early stages of the repair process.

Example 11

The sciatic nerve of adult rats was transected and the proximal end inserted 2mm into a silicone guide tube taking the form of the tube shown in Fig. 7. As in the experiment hereinabove described with reference to Fig. 7, a single central guiding filament (10-0

monofilament nylon) was stitched through the proximal sciatic nerve end to extend into the lumen, the lumen was filled with PBS and melagatran then infused locally with the aid of an implanted osmotic minipump. The distal end of the transected sciatic nerve was positioned among the thigh muscles at a distance from the silicone tube and could thus not interfere with the regenerate.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution and direction of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were arranged parallel to the central guiding filament and very few diverged except in the immediate vicinity of the proximal nerve end. There was no axonal aberration and hardly any loops at all except for a few at the proximal nerve end. The Schwann cells were mainly arranged parallel to the central guiding filament. Fibroblasts formed an enclosing perineurium-like structure. There were hardly any macrophages and blood cells outside the regenerate.

Excellent regeneration of coherent axons was thus achieved due to the nerve tissue regenerate following the path laid by a coherent fibrin network formed in the early stages of the repair process.

Example 12

The set up for this Example was the same as that in Example 11 other than that streptokinase (Hoescht) was infused locally with the aid of a miniosmotic pump.

Results similar to those described above in Example 11 were obtained. However, the osmotic minipump was changed every second day to attain sufficient streptokinase activity during an 8 day period.

Example 13

The set up for this Example was the same as that in Example 11 other than that the recombinant human plasminogen activator (hrtPA) Actilyse ® (Boehringer Ingelheim) was infused locally with the aid of an osmotic minipump.

Results similar to those described above in Example 11 were also obtained.

Example 14

The set up for this Example was the same as that in Example 11 other than that urokinase was infused locally with the aid of an osmotic minipump.

Results similar to those described above in Example 11 were obtained.

Example 15

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. As in Example 3, the proximal and distal nerve ends were inserted 2 mm into a silicone tube prefilled with buffered saline and having through its centre a guiding filament, e.g. a suture thread. The gap between the nerve ends was 10 mm. The silicone tube was sutured to the perineurium of the inserted nerves with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

In this case, though, an osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Hirudin, (Sigma)) was implanted subcutaneously in the back of the animal and the outlet of the pump connected via a tube to the mid portion of the silicone tube enclosing the transected nerve for local delivery of Hirudin into the gap in the manner shown in Fig. 7.

The regenerate formed in the gap was after 2 and 4 weeks examined with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised by immunohistochemistry. The axons were numerous and highly coherent, showing only minor aberrations, and were rarely arranged as loops. The Schwann cells were identified in the central parts of the regenerate, and showed a high degree of coherence as well. Fibroblasts formed an enclosing perineurium-like structure. Rare macrophages were noticed in the gap region.

Excellent regeneration of coherent axons across a 10 mm gap was thus achieved.

Example 16

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. As in Example 7 the proximal and distal nerve ends were inserted 2 mm into a silicone tube prefilled with buffered saline but having no guiding filament, e.g. a suture thread, in the centre of the silicone tube. The gap between the nerve ends was 10 mm. The silicone tube was sutured to the perineurium of the inserted nerves with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

In contrast to Example 7, an osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Hirudin (Sigma)) was implanted subcutaneously in the back of the animal and the outlet of the pump connected via a tube to the mid portion of the silicone tube enclosing the transected nerve for local delivery into the gap as shown in Fig. 7.

The nerve and the tube with its gap were examined after 2 and 4 weeks with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised by immunohistochemistry with the aid of antibodies to neurofilaments (N 0142 & N 5389, Sigma) and to the neuroglial protein S-100 (S 2644, Sigma; Z 0311, Dakopatts). There was

no regenerate bridging the gap. No axons or Schwann cells traversed the gap. Scattered amorphous protein strands and cells could be recognised in the gap fluid.

Nerve regeneration was thus blocked in absence of a guiding structure, such as a filament covered with a fibrin network.

Example 17

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. As in Example 16, the proximal and distal nerve ends were inserted 2 mm into a silicone tube prefilled with buffered saline but not having a guiding filament, e.g. a suture thread, in the centre of the silicone tube. The gap between the nerve ends was 10 mm. The silicone tube was sutured to the perineurium of the inserted nerves with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

In contrast to Example 16, an osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Hirudin (Sigma)) was implanted in the peritoneal cavity and the outlet of the pump left open for systemic delivery of Hirudin via the peritoneal cavity and the blood system.

The nerve and the tube with its gap were examined after 2 and 4 weeks with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised by immunohistochemistry with the aid of antibodies to neurofilaments (N 0142 & N 5389, Sigma) and to the neuroglial protein S-100 (S 2644, Sigma; Z 0311, Dakopatts). There was no regenerate bridging the gap. No axons or Schwann cells traversed the gap. Scattered amorphous protein strands and cells could be recognised in the gap fluid.

Nerve regeneration was thus blocked in absence of a mechanical guiding structure, such as a filament covered with a fibrin network.

Example 18

In this Example parts of the Achilles tendon of adult rats were transected and the proximal and distal ends introduced 1mm into a silicone tube taking the form of that shown in Figs 1 to 6. A single central guiding filament (10-0 monofilament nylon) was provided to extend through the lumen of the tube to connect the proximal and distal tendon stumps. PBS or melagatran were then infused systemically with the aid of an osmotic minipump.

After 3 weeks more parallel fibroblasts and a higher degree of ordered coherence of the collagen fibres could be recognised in the tubes treated with melagatran as compared to the tubes treated with PBS.

Example 19

The Achilles tendon was exposed unilaterally on adult rats and a portion of it transected. As in Example 3, the transected and isolated proximal and distal tendon ends were inserted 2 mm into a silicone tube prefilled with buffered saline and having through its centre a guiding filament, e.g. a suture thread. The gap between the tendon ends was 4-6 mm. The silicone tube was sutured to the paratenon with 9-0 "atraumatic" ophthalmic sutures (Ethicon). An osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Melagatran, Astra Hässle) was implanted subcutaneously in the back of the animal and the outlet of the pump connected via a tube to the mid portion of the silicone tube enclosing the transected tendon for local delivery of Melagatran into the gap as shown in Fig. 7.

The regenerate formed in the silicone tube was examined after 2 and 4 weeks with regard to the distribution, direction and coherence of collagen fibres and cells. The gap was filled with collagen fibres showing high degrees of coherence. Randomly organised collagen structures were rare. Fibroblasts and vascular wall cells were common while macrophages and inflammatory cells were rare. A paratenon-like structure could be recognised.

Good regeneration of the tendon was thus achieved.

Example 20

The Achilles tendon was exposed unilaterally on adult rats and a portion of it transected. As in Example 7, the transected and isolated proximal and distal tendon ends were inserted 2 mm into a silicone tube prefilled with buffered saline but lacking a guiding filament such as a suture thread inserted in the centre of the silicone tube. The gap between the tendon ends was 4 - 6 mm. The silicone tube was sutured to the paratenon with 9-0 "atraumatic" ophthalmic sutures (Ethicon).

An osmotic minipump (Alza 2002, volume = 220 µL; pumping rate = 0.5 µL/h; Alza Corp.; Palo Alto, CA, USA; prefilled with a solution of the thrombin inhibitor Melagatran, (Astra Hässle)) was implanted subcutaneously in the back of the animal and the outlet of the pump connected via a tube to the mid portion of the silicone tube enclosing the transected nerve for local delivery of Melagatran into the gap.

The regenerate formed in the silicone tube was examined after 2 and 4 weeks with regard to the distribution, direction and coherence of collagen fibres and cells. The gap was incompletely filled with randomly organised collagen filaments, highly variable in size and direction. There was a lack of good coherence. Randomly organised collagen structures thus dominated. Fibroblasts and vascular wall cells were common while macrophages and inflammatory cells were rare.

Deficient repair of the tendon was thus achieved.

Example 21

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. The proximal and distal nerve ends were then each inserted 2 mm into a 10mm silicone tube (inner diameter 1.8mm) of the type shown in Figs 1 to 6 to leave a gap in the lumen between the nerve ends of 6 mm prefilled with a homogeneous 1% agar gel. The silicone tube was sutured to the epineurium of the inserted nerves ends with 10-0 "atraumatic" monofilament Ethilone® sutures (Ethicon).

The nerve and the tube with its gap were examined after 2 and 4 weeks with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised by immunohistochemistry with the aid of antibodies to neurofilaments (N 0142 & N 5389, Sigma) and to the neuroglial protein S-100 (S 2644, Sigma; Z 0311, Dakopatts).

The tissue filling the gap between the nerve ends and the gel, which lacked channels, was composed of highly irregular connective tissue with loops of cells and filaments. The Schwann cells were randomly aligned. There was no distinct minifascicles or bundles of neurites. The axons showed extensive aberration and formed part of neuroma-like structures. Randomly organized axons were observed between the inner surface of the silicone tube and the agar gel.

A positive pinch test was elicited after 4 weeks at a distance of 15 mm in one out of 4 animals, the other 3 being negative.

Nerve regeneration was thus blocked in absence of a mechanical guiding structure.

Example 22

The sciatic nerve was exposed unilaterally on the mid thigh of adult rats and transected. The proximal and distal nerve ends were then each inserted 2 mm into a 10mm silicone tube (inner diameter 1.8mm) to leave a gap in the lumen between the nerve ends of 6 mm prefilled with a 1% agar gel having 3 or 5 longitudinal channels of nominal diameter of 0.4mm formed by temporary insertion of filaments during the gelation procedure, for example as shown in Fig. 8. The silicone tube was sutured to the epineurium of the inserted nerves ends with 10-0 "atraumatic" monofilament Ethilone® sutures (Ethicon).

The nerve and the tube with its gap were examined after 2 and 4 weeks with regard to the distribution, direction and coherence of the axons and of the Schwann cells, as visualised for example by immunohistochemistry with the aid of antibodies to neurofilaments (N 0142 & N 5389, Sigma) and to the neuroglial protein S-100 (S 2644, Sigma; Z 0311, Dakopatts).

The interfaces between the nerve ends and the gel and between the gel and the enclosing silicone tube were filled with tissue, rebuilt and restructured in a regular manner over time. Macroscopic inspection after 1 week revealed that a fibrin network was converging towards the opening of each one of the channels, proximally as well as distally.

Scanning electron microscopy proved the presence of a coherent axially aligned fibrin-cell-network, rich in platelets, converging towards the openings of the channels and extending through them. After 2 and most evidently after 4 weeks axons and Schwann cells were identified in the interface between the nerve ends and the gel and in the channels delimited by granulation tissue and its blood vessels. The cells entering the gap zone followed the path of the cables of fibrin and platelets. Only a minor inflammatory cell reaction was noticed in the gel channels and at the interface between the agar and the enclosing silicone tube.

The nerve tissue bridging the conduit gap from the proximal to the distal nerve end through the intermediate channels were arranged in minifascicles, mainly composed of axons and

Schwann cells enclosed by a thin perineurium. The axons and the Schwann cells were arranged in distinct fascicle bundles, bridging between the nerve ends through the channels. In addition, randomly orientated Schwann cells could be recognised between the fairly coherent cell bundles in the gap region. The number of axons per channel increased by a factor in the order of at least 3 from 2 weeks to 4 weeks. The perineural connective tissue enclosing the minifascicles was less abundant than that observed in the regenerates from PBS filled tubes, e.g. Example 1.

Axons were as well observed along the interface between the gel and the tube. The vast majority of these axons were randomly orientated, lacking the high degree of axonal coherence demonstrable in the gel channels.

The axons in the 5 channel system showed good coherence even when entering the distal nerve.

Positive pinch tests were elicited after 4 weeks at a greater distance than when homogeneous agar gel filled the gap in the lumen of the tube between the nerve ends, with the 5-channel system eliciting positive pinch tests at a greater distance than the 3-channel system. Moreover, almost twice the number of coherent axons advancing at about twice the rate was achieved as compared to the homogenous gel set up in Example 21 and also the set up in Example 1.

Excellent regeneration across the gap was thus achieved

Similar results to those achieved in the Examples above which used melagatran and streptokinase have also been obtained in experiments on regenerating entubated abdominal aponeurose and thigh skeletal muscles, i.e. treatment with melagatran and streptokinase resulted in better structural order in the regenerated tissue as compared to that after infusion of PBS subcutaneously. Furthermore, corresponding experiments conducted with inogatran, heparin, the recombinant human plasminogen activator Actilyse® and urokinase have

produced similar improvements in the repair and regeneration of a wound area in an organised tissue structure.

It can therefore been seen that use of an encasement structure, such as the guide tubes of the Examples, with mechanical guide means for the tissue regenerate, such as the central guide filaments of the Examples, in combination with a fibrin network formation inhibiting agent, such as a fibrinolytic agents and/or a thrombin inhibitor, causes an improvement in the regeneration of the injured structure when compared to the hitherto proposed systems. An improvement is also forthcoming when a gel which presents guides channels is provided in a wound area encased by an encasement structure.

In accordance with the present invention, the materials which may be used as a carrier matrix for the fibrin network formation inhibiting agent includes any material or system in which the agent can be inserted into or conveyed into the encased wound area such as any biocompatible material into which the agent can be suspended, dissolved or released from. Such carrier materials may include, but are not limited to, collagen, methylcellulose gel, chitosan and other polysaccharides, fibrin or other proteins, extracellular matrix materials such a Matrigel™ (Collaborative Research, Inc., Waltham, MA, USA), Biomatrix ™ (Biomedical Technologies, Inc., Stoughton, MA) or other related materials. The carrier may also comprise saline, water or, as in the Examples described above, buffered solutions which may be delivered to the encased wound area using a continuous delivery system, such as an osmotic mini-pump or externally accessible catheter connected to the device for continuous delivery. The fibrin network formation inhibiting agent could also be immobilised in the encasement structure.

The present invention also provides for growth-stimulating agents to be placed or delivered into the encased wound area. Such agents include trophic, chemotactic, mitogenic, or similar substances, or combinations or mixtures thereof, which are capable of stimulating growth, directly or indirectly. Most preferably, these agents include insulin like growth factors-I, insulin-like growth factors-II, platelet derived growth factors, interleukines,

cytokines, fibroblast growth factors, transforming growth factors-ß, transforming growth factors-a, epidermal growth factors, brain-derived neurotrophic factors, neurotrophines, ciliary neurotrophic factors, EGF and glial growth factors. Therapeutic agents may also include whole cells or their parts which may be delivered to the encased wound area. These include Schwann cells, endothelial cells, fibroblasts, monocytes, macrophages, inflammatory cells or genetically altered cells or mixtures thereof.

As an example, the growth-stimulating agent may be incorporated in the carrier matrix for the fibrin network formation inhibiting agent or in its own carrier, for example genetically altered or unaltered cells capable of delivering growth-stimulating agents may be incorporated as a delivery system for the growth-stimulating agents to the encased injured structure. The growth-stimulating agent may also be immobilised in the encasement structure for slow release thereof.

CLAIMS

1. A system for promoting growth of tissue regenerate into a wound area in an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction comprising:

an encasement structure adapted in use to be implanted in the living human or animal body to encase the wound area,

mechanical guide means for the tissue regenerate adapted in use to be disposed in the encased wound area so as to extend in the predetermined direction, and

a fibrin network formation inhibiting agent administrable to the wound surface of the encased wound area.

2. A system according to claim 1, characterised in that the fibrin network formation inhibiting agent comprises a thrombin inhibitor.

3. A system according to claim 2, characterised in that the thrombin inhibitor is a low molecular weight peptide-based thrombin inhibitor.

4. A system according to claim 3, characterised in that the thrombin inhibitor is a gatran.

5. A system according to claim 4, characterised in that the thrombin inhibitor is melagatran or inogatran.

6. A system according to claim 2, characterised in that the thrombin inhibitor is a bisulphated polysaccharide or oligosaccharide such as a chondroitin sulphate, a dextron sulphate, a keratan sulphate, a dermatan sulphate, a heparan sulphate or heparin.

7. A system according to claim 2, characterised in that the thrombin inhibitor is a hirudin, a biosynthetic analogue of hirudin, a fragment of hirudin such as a fragment

consisting of at least the last 8 C-terminal amino acids of the known sequence in hirudin or the protein NAPc2.

8. A system according to claim 1, characterised in that the fibrin network formation inhibiting agent comprises a fibrinolytic agent.

9. A system according to claim 8, characterised in that the fibrinolytic agent is a plasminogen activator (tPA), streptokinase or urokinase.

10. A system according to claim 8, characterised in that the fibrinolytic agent is a recombinant human plasminogen activator (hrtPA) such as Actilyse®.

11. A system according to claim 1, characterised in that the fibrin network formation inhibiting agent comprises a Factor X inhibitor.

12. A system according to claim 1, characterised in that the fibrin network formation inhibiting agent comprises a trypsin inhibitor.

13. A system according to claim 1, characterised in that the fibrin network formation inhibiting agent comprises a protease inhibitor.

14. A system according to any one of claims 1 to 13, characterised in that the fibrin network formation inhibiting agent is immobilised to the inner surface of the encasement structure which in use faces the wound area.

15. A system according to any one of claims 1 to 13, characterised in that the fibrin network formation inhibiting agent is in solution and that the system further comprises a pump for administering the fibrin network formation inhibiting agent to the encased wound area.

16. A system according to claim 15, characterised in that the pump is an osmotic minipump.

17. A system according to claim 15 or 16, characterised in that the pump is adapted to be implanted subcutaneously in the living human or animal body.

18. A system according to any one of claims 1 to 13, characterised in that the fibrin network formation inhibiting agent is incorporated in a matrix material for disposal or delivery to the encased wound area.

19. A system according to claim 18, characterised in that the matrix material is formed of a material comprising a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, an agar gel, a hydrogel such as methylcellulose gel, Matrigel®, Biomatrix I®, water, saline, phosphate buffered saline, a lipid or a protein such as collagen.

20. A system according to claim 1, characterised in that the fibrin network formation inhibiting agent is adapted to be administered to the encased wound area systemically or locally.

21. A system according to any one of the preceding claims, characterised in that the encasement structure is a patch for a crush wound area or the like of the organised tissue structure.

22. A system according to any one of claims 1 to 20, characterised in that the encasement structure is a tube having an open end adapted to receive the wound surface and that the mechanical guide means is adapted in use to extend in the predetermined direction in the lumen of the tube.

23. A system according to claim 22, characterised in that the tube comprises an outer continuous tube element and an inner tube element which is formed of a plurality of longitudinally spaced apart tube sections.

24. A system according to claim 22 or 23, characterised in that the wound surface of the wound area is a first wound surface, that the open end of the tube is a first open end, that the tube has a second open end adapted to receive a second wound surface of the wound area and that the mechanical guide means is adapted in use to extend in the lumen of the tube between the first and second open ends in the predetermined direction.

25. A system according to claim 24, characterised in that the system is for promoting growth of tissue regenerate across a gap between the severed or transected free ends of an organised tissue structure such as a nerve, tendon, skeletal muscle or ligament and that the open ends of the tube are each adapted to receive one of the severed or transected free ends.

26. A system according to any one of the preceding claims, characterised in that the encasement structure is of a biocompatible material.

27. A system according to any one of the preceding claims, characterised in that the encasement structure is of a biodegradable material.

28. A system according to any one of claim 1 to 26, characterised in that the encasement structure is of a non-biodegradable material.

29. A system according to any one of claims 1 to 25 , characterised in that the encasement structure is constructed from a polysaccharide.

30. A system according to claim 29, characterised in that the encasement structure is constructed from a material comprising a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

31. A system according to any one of claims 1 to 25, characterised in that the encasement structure is constructed of a material comprising collagen or other protein complexes.

32. A system according to any one of claims 1 to 25, characterised in that the encasement structure is constructed from a material comprising a polymer or copolymer.

33. A system according to claim 32, characterised in that the encasement structure is constructed of a material comprising polylactic acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, polyglucuronic acid, or poly-N-acetylglucosamine or copolymers thereof.

34. A system according to claim 32, characterised in that the encasement structure is constructed of a material comprising a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid

35. A system according to claim 28, characterised in that the encasement structure is constructed from silicone or ethylene-vinyl acetate.

36. A system according to any one of the preceding claims, characterised in that the mechanical guide means is supported or presented by the inner surface of the encasement structure which in use faces the wound area.

37. A system according to claim 36, characterised in that the mechanical guide means and the encasement structure are integrally formed as an implantable body.

38. A system according to any one of the preceding claims, characterised in that the mechanical guide means takes the form of guide channels in the encased wound area.

39. A system according to claim 38 as appendant to any one of claims 1 to 20, characterised in that the encasement structure when implanted is a tube-like structure having a transverse spiral cross-section formed for example by rolling up a planar membrane and that the guide channels are defined by the longitudinally extending spaces presented by the spiral cross-section.

40. A system according to claim 38, characterised in that the mechanical guide means takes the form of a gel structure which is provided with one or more guide channels therethrough, the gel structure adapted in use to be disposed in the encased wound area such that the guide channels extend in the predetermined direction.

41. A system according to claim 38, 39 or 40, characterised in that the system is for promoting the growth of nerve tissue regenerate and that the or each guide channel has a cross-sectional dimension in the range of 50μm-1mm.

42. A system according to claim 41, characterised in that the or each guide channel has a cross-sectional dimension in the range of 150-500μm.

43. A system according to claim 40, characterised in that the gel structure is formed from agar, a hydrogel such as methylcellulose gel, albumin or other proteins which can be formed into gel, a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, a lipid which can be formed into a gel, Matrigel® or Biomatrix I®.

44. A system according to any one of claims 1 to 37 , characterised in that the mechanical guide means comprises one or more guide filaments or fibres adapted in use to extend across the encased wound area in the predetermined direction.

45. A system according to claim 44, characterised in that the mechanical guide means comprises one or more monofilaments, multifilaments or woven/non-woven fibres.

46. A system according to claim 44 or 45, characterised in that the or each guide filament or fibre is of a biocompatible material.

47. A system according to claim 44, 45 or 46, characterised in that the or each guide filament or fibre is formed from a biodegradable material.

48. A system according to claim 44, 45 or 46, characterised in that the or each guide filament or fibre is formed from a non-biodegradable material.

49. A system according to claim 44 or 45 characterised in that the or each guide filament or fibre is formed from a material comprising a polysaccharide.

50. A system according to claim 49, characterised in that the or each guide filament or fibre is formed from a material comprising a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

51. A system according to claim 44 or 45, characterised in that the or each guide filament or fibre is formed from a material comprising a polymer or copolymer.

52. A system according to claim 51, characterised in that the or each guide filament or fibre is formed from polylactic-acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, poly-N-acetylglucosamine or copolymers thereof such as for example a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid.

53. A system according to claim 44 or 45, characterised in that the or each guide filament or fibre is formed from collagen or other protein complexes.

54. A system according to claim 44, characterised in that the mechanical guide means comprises one or more suture filaments.

55. A system according to claim 54, characterised in that the or each suture filament is formed from vicryl, catgut, polyamid, chitin or nylon.

56. A system according to claim 48, characterised in that the or each guide filament or fibre is formed from silicone.

57. A system according to any one of the preceding claims, characterised in that the system further comprises a growth factor or mixture of growth factors for administration to the encased wound area.

58. A system according to claim 57, characterised in that the growth factor is immobilised to the inner surface of the encasement structure.

59. A system according to claim 57 or 58, characterised in that the growth factor comprises insulin-like growth factors-I, insulin-like growth factors-II, platelet derived growth factors, fibroblast growth factors, transforming growth factors-β, transforming growth factors-α, neurotrophines, ciliary neurotrophic factors, EGF or glial growth factors.

60. A system according to claim 59, characterised in that the growth factor comprises Schwann cells, endothelial cells, fibroblasts, macrophages or inflammatory cells or genetically altered cells which can express a growth factor.

61. A system according to claim 1, characterised in that the system is for promoting the growth of tissue regenerate in a wound area in a nerve, tendon, ligament, joint capsule, cartilage, bone, aponeurose or skeletal muscle.

62. An implantable device for promoting growth of tissue regenerate into a wound area in an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction comprising:

an outer encasement structure which when the device is implanted in the living human or animal body encases the wound area, and

an inner gel structure provided with one or more guide channels for the tissue regenerate which when the device is implanted is disposed in the encased wound area such that the guide channels extend in the predetermined direction.

63. A device according to claim 62, characterised in that the encasement structure is a patch for a crush wound area or the like of the organised tissue structure.

64. A device according to claim 62, characterised in that the encasement structure is a tube having an open end adapted to receive the wound surface with the or each guide channel extending in the lumen of the tube in the predetermined direction.

65. A device according to claim 64, characterised in that the wound surface of the wound area is a first wound surface, that the open end of the tube is a first open end, that the tube has a second open end adapted to receive a second wound surface of the wound area and that the or each guide channel extends in the lumen of the tube between the first and second open ends in the predetermined direction.

66. A device according to claim 65, characterised in that the device is for promoting growth of tissue regenerate across a gap between the severed or transected free ends of an organised tissue structure such as a nerve, tendon, skeletal muscle or ligament and that the open ends of the tube are each adapted to receive one of the severed or transected free ends.

67. A device according to any one of claims 62 to 66, characterised in that the encasement structure is of a biocompatible material.

68. A device according to any one of claims 62 to 67, characterised in that the encasement structure is of a biodegradable material.

69. A device according to any one of claims 62 to 67, characterised in that the encasement structure is of a non-biodegradable material.

70. A device according to any one of claims 62 to 66 , characterised in that the encasement structure is constructed from a material comprising a polysaccharide.

71. A device according to claim 70, characterised in that the encasement structure is constructed from a material comprising a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

72. A device according to any one of claims 62 to 66, characterised in that the encasement structure is constructed of a material comprising collagen or other protein complexes.

73. A device according to any one of claims 62 to 66, characterised in that the encasement structure is constructed from a material comprising a polymer or copolymer.

74. A device according to claim 73, characterised in that the encasement structure is constructed of a material comprising polylactic acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, polyglucuronic acid, or poly-N-acetylglucosamine or copolymers thereof.

75. A device according to claim 73, characterised in that the encasement structure is constructed of a material comprising a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid.

76. A device according to claim 69, characterised in that the encasement structure is constructed from silicone or ethylene-vinyl acetate.

77. A device according to any one of claims 62 to 76, characterised in that the device is for promoting the growth of nerve tissue regenerate and that the or each guide channel has a cross-sectional dimension in the range of 50μm-1mm.

78. A device according to claim 77, characterised in that the or each guide channel has a cross-sectional dimension in the range of 150-500μm.

79. A device according to any one of claims 62 to 78, characterised in that the gel structure is formed from agar, a hydrogel such as methylcellulose gel, albumin or other proteins which can be formed into a gel, a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, a lipid which can be formed into a gel, Matrigel® or Biomatrix I®.

80. Use of a system a according to any one of claims 1 to 61 for promoting growth of tissue regenerate in a wound area of an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction.

81. Use of an implantable device according to any one of claims 62 to 79 for promoting growth of tissue regenerate in a wound area of an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction.

82. A method for promoting growth of tissue regenerate in a wound area of an organised tissue structure in a living human or animal body from a wound surface of the wound area in a predetermined direction comprising the steps of:

encasing the wound area with an encasement structure,

providing mechanical guide means for the tissue regenerate in the encased wound area such that the mechanical guide means extends in the predetermined direction, and

administering a fibrin network formation inhibiting agent to the encased wound area.

83. A method according to claim 82, characterised in that the fibrin network formation inhibiting agent comprises a thrombin inhibitor.

84. A method according to claim 83, characterised in that the thrombin inhibitor is a low molecular weight peptide-based thrombin inhibitor.

85. A method according to claim 84, characterised in that the thrombin inhibitor is a gatran.

86. A method according to claim 85, characterised in that the thrombin inhibitor is melagatran or inogatran.

87. A method according to claim 83, characterised in that the thrombin inhibitor is a bisulphated polysaccharide or oligosaccharide such as a chondroitin sulphate, a dextron sulphate, a dermatan sulphate, a keratan sulphate, a heparan sulphate or heparin.

88. A method according to claim 83, characterised in that the thrombin inhibitor is a hirudin, a biosynthetic analogue of hirudin, a fragment of hirudin such as a fragment consisting of at least the last 8 C-terminal amino acids of the known sequence in hirudin or the protein NAPc2.

89. A method according to claim 82, characterised in that the fibrin network formation inhibiting agent comprises a fibrinolytic agent.

90. A method according to claim 89, characterised in that the fibrinolytic agent is a plasminogen activator (tPA), streptokinase or urokinase

91. A method according to claim 89, characterised in that the fibrinolytic agent is a recombinant human plasminogen activator (hrtPA) such as Actilyse®.

92. A method according to claim 82, characterised in that the fibrin network formation inhibiting agent comprises a Factor X inhibitor.

93. A method according to claim 82, characterised in that the fibrin network formation inhibiting agent comprises a trypsin inhibitor.

94. A method according to claim 82, characterised in that the fibrin network formation inhibiting agent comprises a protease inhibitor.

95. A method according to any one of claims 82 to 94, characterised in that the fibrin network formation inhibiting agent is immobilised to the inner surface of the encasement structure which in use faces the wound area.

96. A method according to any one of the claims 82 to 94, characterised in that the fibrin network formation inhibiting agent is in solution and that the method further comprises the step of providing a pump for administering the fibrin network formation inhibiting agent to the encased wound area.

97. A method according to claim 96, characterised in that the pump is an osmotic minipump.

98. A method according to claim 96 or 97, characterised in that the pump is implanted subcutaneously in the living human or animal body.

99. A method according to any one of claims 82 to 94, characterised in that the fibrin network formation inhibiting agent is incorporated in a matrix material for disposal or delivery to the encased wound area.

100. A method according to claim 99, characterised in that the matrix material is formed of a material comprising a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, an agar gel, a hydrogel such as methylcellulose gel, Matrigel®, Biomatrix I®, water, saline, phosphate buffered saline, a lipid or a protein such as collagen.

101. A method according to claim 82, characterised in that the fibrin network formation inhibiting agent is administered to the encased wound area systemically or locally.

102. A method according to any one of the claims 82 to 101, characterised in that the encasement structure is a patch for a crush wound area or the like of the organised tissue structure.

103. A method according to any one of claims 82 to 101, characterised in that the encasement structure is a tube having an open end in which the wound surface is received and that the mechanical guide means extends in the predetermined direction in the lumen of the tube.

104. A method according to claim 103, characterised in that the tube comprises an outer continuous tube element and an inner tube element which is formed of a plurality of longitudinally spaced apart tube sections.

105. A method according to claim 103 or 104, characterised in that the wound surface of the wound area is a first wound surface, that the open end of the tube is a first open end, that the tube has a second open end in which a second wound surface of the wound area is received and that the mechanical guide means extends in the lumen of the tube between the first and second open ends in the predetermined direction.

106. A method according to claim 105, characterised in that the method is for promoting growth of tissue regenerate across a gap between the severed or transected free

ends of an organised tissue structure such as a nerve, tendon, skeletal muscle or ligament and that the open ends of the tube each receive one of the severed or transected free ends.

107. A method according to any one of claims 82 to 106, characterised in that the encasement structure is of a biocompatible material.

108. A method according to any one of claims 82 to 107, characterised in that the encasement structure is of a biodegradable material.

109. A method according to any one of claims 82 to 107, characterised in that the encasement structure is of a non-biodegradable material.

110. A method according to any one of claims 82 to 106 , characterised in that the encasement structure is constructed from a material comprising a polysaccharide.

111. A method according to claim 110, characterised in that the encasement structure is constructed from a material comprising a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

112. A method according to any one of claims 82 to 106, characterised in that the encasement structure is constructed of a material comprising collagen or other protein complexes.

113. A method according to any one of claims 82 to 106, characterised in that the encasement structure is constructed from a material comprising a polymer or copolymer.

114. A method according to claim 113, characterised in that the encasement structure is constructed of a material comprising polylactic acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, polyglucuronic acid or poly-N-acetylglucosamine or copolymers thereof.

115. A method according to claim 113, characterised in that the encasement structure is constructed of a material comprising a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid.

116. A method according to claim 109, characterised in that the encasement structure is constructed from silicone or ethylene-vinyl acetate.

117. A method according to any one of claims 82 to 116, characterised in that the mechanical guide means is supported or presented by the inner surface of the encasement structure which faces the wound area.

118. A method according to claim 117, characterised in that the mechanical guide means and the encasement structure are integrally formed.

119. A method according to any one of claims 82 to 118, characterised in that the mechanical guide means takes the form of guide channels in the encased wound area.

120. A method according to claim 119 as appendant to any one of claims 82 to 101, characterised in that the encasement structure is a tube-like structure having a transverse spiral cross-section formed for example by rolling up a planar membrane and that the guide channels are defined by the longitudinally extending spaces presented by the spiral cross-section.

121. A method according to claim 119, characterised in that the mechanical guide means takes the form of a gel structure which is provided with one or more guide channels therethrough and disposed in the encased wound area such that the guide channels extend in the predetermined direction.

122. A method according to claim 119, 120 or 121, characterised in that the method is for promoting the growth of nerve tissue regenerate and that the or each guide channel has a cross-sectional dimension in the range of 50μm-1mm.

123. A method according to claim 122, characterised in that the or each guide channel has a cross-sectional dimension in the range of 150-500μm.

124. A method according to claim 121, characterised in that the gel structure is formed from agar, a hydrogel such as methylcellulose gel, albumin or other proteins which can be formed into a gel, a polysaccharide such as a chitosan or a hyaluronan such as hyaluronic acid, a lipid which can be formed into a gel, Matrigel® or Biomatrix I® .

125. A method according to any one of claims 82 to 118 , characterised in that the mechanical guide means comprises one or more guide filaments or fibres extending across the encased wound area in the predetermined direction.

126. A method according to claim 125, characterised in that the mechanical guide means comprises one or more monofilaments, multifilaments or woven/non-woven fibres.

127. A method according to claim 125 or 126, characterised in that the or each guide filament or fibre is of a biocompatible material.

128. A method according to claim 125, 126 or 127, characterised in that the or each guide filament or fibre is formed from a biodegradable material.

129. A method according to claim 125, 126 or 127, characterised in that the or each guide filament or fibre is formed from a non-biodegradable material.

130. A method according to claim 125 or 126 characterised in that the or each guide filament or fibre is formed from a polysaccharide.

131. A method according to claim 130, characterised in that the or each guide filament or fibre is formed from a chitosan, heparin, a heparanoid or a hyaluronan such as hyaluronic acid.

132. A method according to claim 125 or 126, characterised in that the or each guide filament or fibre is formed from a polymer or copolymer.

133. A method according to claim 132, characterised in that the or each guide filament or fibre is formed from polylactic acid, polyhydroxybutyric acid, polyglycolic acid, permselective polytetraethylene, poly-N-acetylglucosamine or copolymers thereof such as for example a copolymer of polyhydroxybutyric acid and hydroxyvaleric acid.

134. A method according to claim 125 or 126, characterised in that the or each guide filament or fibre is formed from collagen or other protein complexes.

135. A method according to claim 125 or 126, characterised in that the mechanical guide means comprises one or more suture filaments.

136. A method according to claim 135, characterised in that the or each suture filament is formed from vicryl, catgut, polyamid, chitin or nylon.

137. A method according to claim 129, characterised in that the or each guide filament or fibre is formed from a material comprising silicone.

138. A method according to any one of claims 82 to 137, characterised in that method further comprises the further step of administering a growth factor to the encased wound area.

139. A method according to claim 138, characterised in that the growth factor is immobilised to the inner surface of the encasement structure.

140. A method according to claim 138 or 139, characterised in that the growth factor comprises insulin-like growth factors-I, insulin-like growth factors-II, platelet derived growth factors, fibroblast growth factors, transforming growth factors-β, transforming growth factors-α, neurotrophines, ciliary neurotrophic factors, EGF or glial growth factors.

141. A method according to claim 138 or 139, characterised in that the growth factor comprises Schwann cells, endothelial cells, fibroblasts, macrophages or inflammatory cells or genetically altered cells which can express a growth factor.

142. A method according to claim 82, characterised in that the method is for promoting the growth of tissue regenerate in a wound area in a nerve, tendon, ligament, joint capsule, cartilage, bone, aponeurose or skeletal muscle.

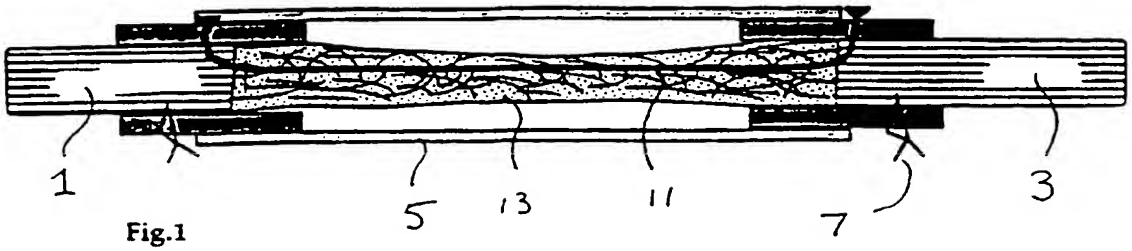

Fig.1

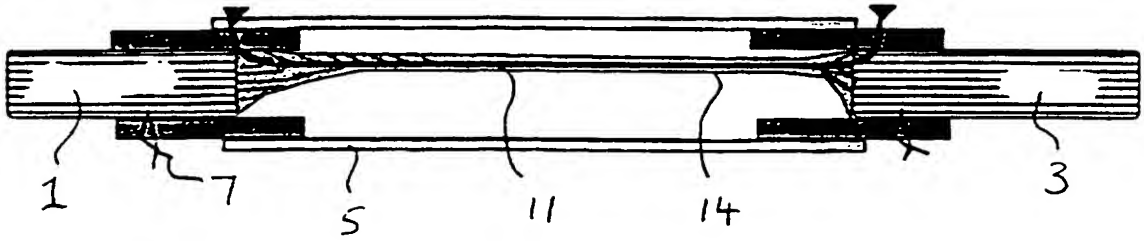

Fig.2

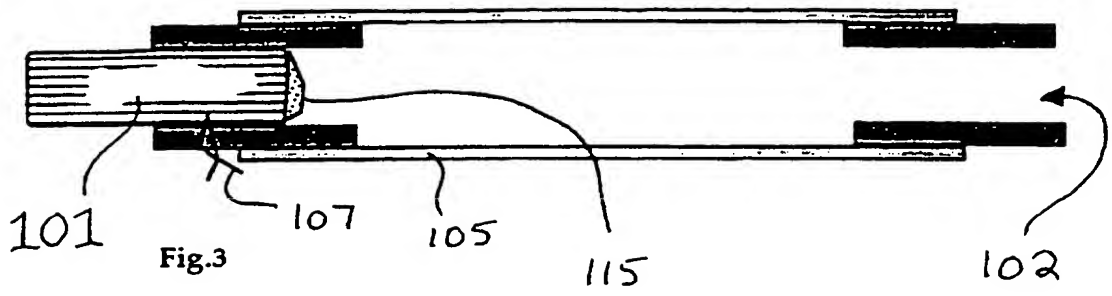

Fig.3

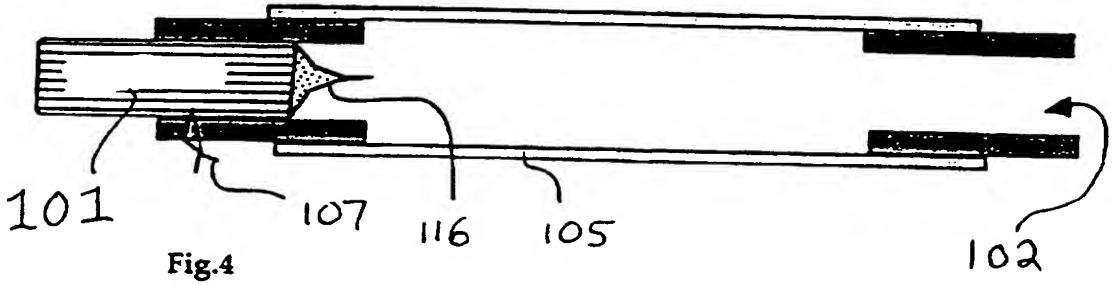

Fig.4

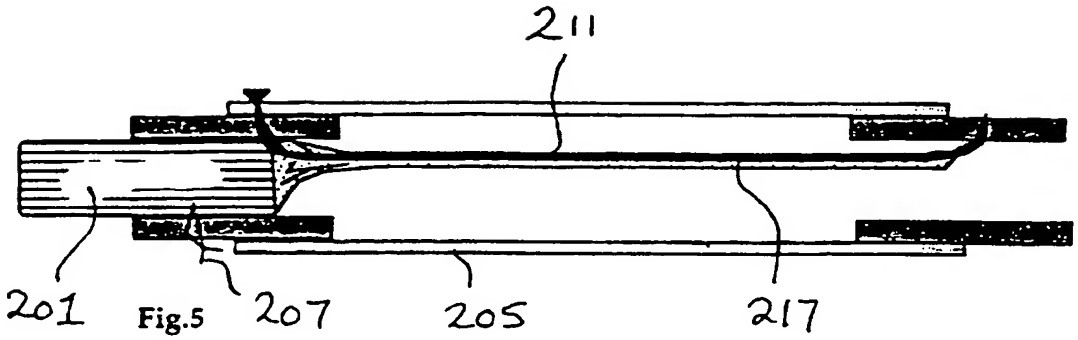

Fig.5

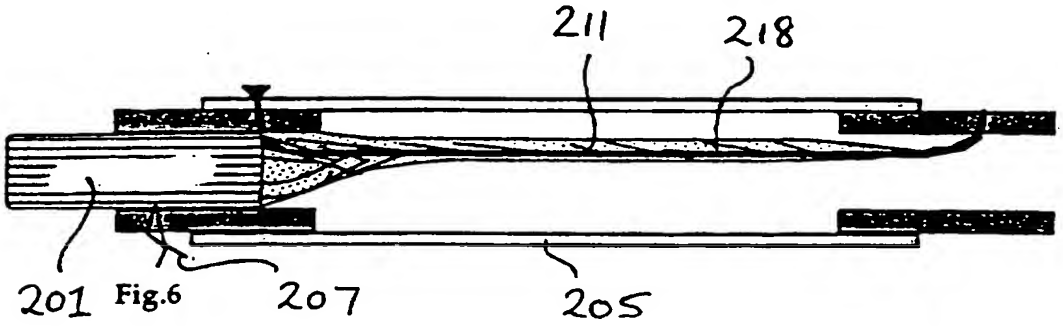

Fig.6

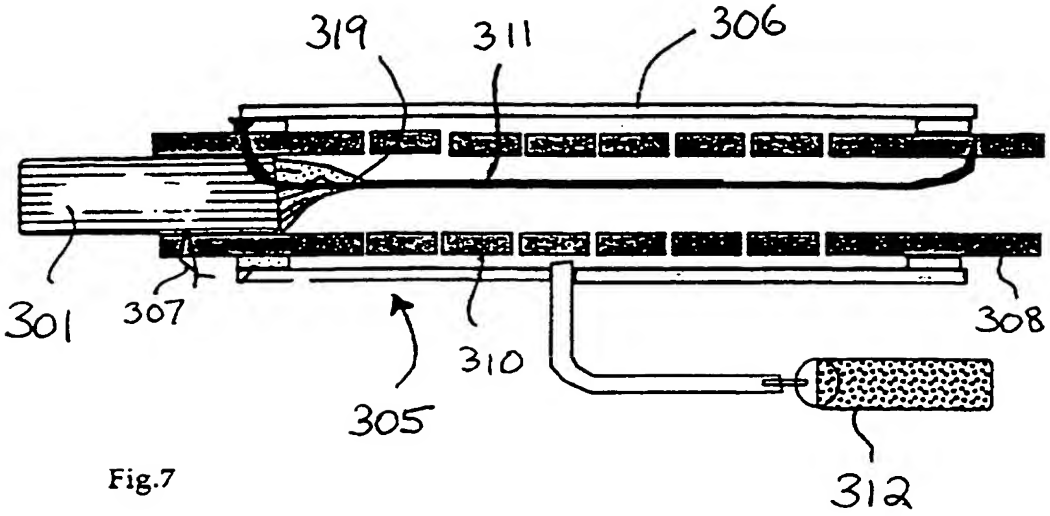

Fig.7

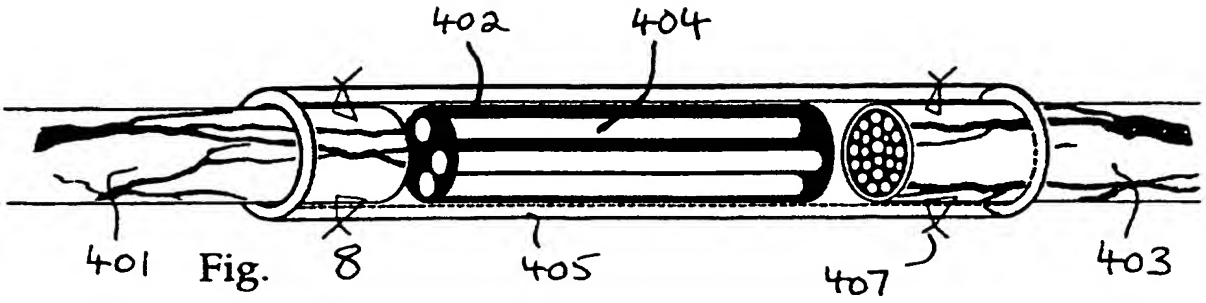

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00565

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 5/06, A61L 31/00, A61F 2/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61F, A61L, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, WPI, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9602286 A1 (CYTOTHERAPEUTICS, INC.), 1 February 1996 (01.02.96), see page 12-22 --	1-79
X	US 4963146 A (SHU-TUNG LI), 16 October 1990 (16.10.90), column 10, line 39 - line 47; column 4, line 26 - column 6, line 49, see claim 14 --	1-79
A	US 5292802 A (WOONZA RHEE ET AL), 8 March 1994 (08.03.94) --	1-79
A	WO 9310806 A1 (INSTITUE OF MOLECULAR BIOLOGY, INC.), 10 June 1993 (10.06.93), see pages 14-20 --	1-38,40-61

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
29 August 1997

Date of mailing of the international search report
29-08-1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00565

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9005552 A1 (BROWN UNIVERSITY RESEARCH FOUNDATION), 31 May 1990 (31.05.90), page 4 - page 8; page 10 - page 11	1-79
A	WO 9520359 A1 (INSTITUTE OF MOLECULAR BIOLOGY, INC.), 3 August 1995 (03.08.95), page 4 - page 5; page 11 - page 12, figure 1, claims	1,22-29, 62-79
A	WO 8806871 A1 (ASTRA MEDITEC AB), 22 Sept 1988 (22.09.88)	1-79
A	US 4778467 A (LARRY J. STENSAAS ET AL), 18 October 1988 (18.10.88)	1
A	Dialog Information Services, File 34, SciSearch(R), Dialog accession no. 14505483, HERBERT CB et al: "Effects of fibrinolysis on neurite growth from dorsal-root ganglia cultured in 2-dimensional and 3-dimensional fibrin gels"; Journal of Comparative Neurology, 1996, V365, N3 (FEB 12), P 380-391	1,8-10
A	National Library of Medicine, File Medline, accession no. 95131175, PITTMAN RN: "PC12 cells overexpressing tissue plasminogen activator regenerate neurites to a greater extent and migrate faster than control cells in complex extracellular matrix"; J Neurochem 1995 Feb; 64(2): 566-75	1,8-10

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A	National Library of Medicine, File Medline, accession no. 94065798, NAKAJIMA K et al: "Microglia-derived elastase produces a low-molecular-weight plasminogen that enhances neurite outgrowth in rat neocortical explant cultures": J Neurochem 1993 Dec; 61(6): 21-55-63 --	1,8-10
A	National Library of Medicine, File Medline, accession no. 87102073, HAWKINS RL et al: "Effect of proteases and their inhibitors on neutite outgrowht from neonatal mouse sensory ganglia in culture"; Brain Res 1986 Nov 19; 398(1): 63-70 --	1-7,11-13
A	Dialog Information Services, File 73, EMBASE, Dialog accession no. 9325019, EMBASE no: 9426757, KANAI H.: "Effects of protease inhibitors on neurite outgrowth and survival of cultured neurons"; KITAKANTO MED. J. (Japan), 1994, 44/4 (363-371) --	1-7,11-13
A	Dialog Information Services, File 73, EMBASE, Dialog accession no. 8438762, MCPHERSON J.M.: "The utility of collagen-based vehicles in delivery of growth factors for hard and soft tissue wound repair"; Clin. Mater. (United Kingdom), 1992, 9/3-4 (225-234) --	1-7,14,31
X	US 5019087 A (JOSEPH NICHOLS), 28 May 1991 (28.05.91), column 3 - column 4; column 9, line 14 - line 34, claim 8 and tables --	1-79
X	US 4955893 A (IOANNIS V. YANNAS ET AL), 11 Sept 1990 (11.09.90), see claim 1 and column 3-4 --	62-79

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00565

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0645149 A1 (JOHNSON & JOHNSON MEDICAL, INC.), 29 March 1995 (29.03.95), see figures and claims ---	38-40,62-79
A	WO9522301 A1 (ORGANOGENESIS, INC.), 24 August 1995 (24.08.95), see figure and pages 16-17 --- --------	62-72

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International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 80-142
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 80-142 relate to a method of treatment of the human or animal body by surgery or by therapy practised on the human or animal body/Rule.39.1.(iv). Nevertheless, a search has been executed for claims 80,82-142. The search has been based on the alleged effects of the system.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet.

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

The present application pertains to:

A. A system for regeneration of tissue comprising an encasement structure, guide means for the tissue and a fibrin network formation inhibiting agent according to claims 1-61, and

B. An implantable device for regeneration of tissue comprising an encasement structure and "an inner gels structure provided with one or more guide channels" for the tissue according to claims 62-79.

In the absence of a unifying inventive concept A and B constitute two a priori independent inventions that cannot be adequately handled within one search fee.

INTERNATIONAL SEARCH REPORT

Information on patent family members

06/08/97

International application No.
PCT/SE 97/00565

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